FLORISTIC STUDIES ON LICHENS OF DISTRICT MANDI, HIMACHAL PRADESH, INDIA

A SYNOPSIS

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Introduction:

Lichen thallus is composed of two entirely different organisms (fungus and alga) that have intimate symbiotic relationship. The fungal component is referred to as mycobiont, whereas algal component is referred to as phycobiont. Majority of the mycobionts belong to ascomycetes. Mycobiont constitute 90 percent of lichen thallus and provides shape, structure and colour to it. About 40 genera of algae and cyanobacteria have been found as photobionts (Tschermak-Woess 1988; Budel 1992). Among the photobionts, three genera *Nostoc*, *Trentepohlia* and *Trebouxia* are most common. Lichens are pioneer plants and are capable of colonizing on various substrates under varied environmental conditions.

Lichens are important both ecologically and economically. These play a vital role in biogeochemical weathering of rocks. Lichens are sensitive to environmental pollution and can accumulate heavy metals in thallus (Garty 2001). Lichens are also used in geological dating of rocks and surfaces. Lichens are used in lichenometry for dating surfaces by studying lichen growth rates (Easton 1994). Although accuracy of date estimates is probably no better than 10%, but where alternative dating techniques (e.g. dendrochronology in forested areas) are not applicable, this method has proved valuable in fields such as archaeology and geomorphology (Innes 1988). The method is most useful for dating over the past 500 year and is particularly useful in arctic-alpine environment regions which are above and beyond the tree-line.

Lichen chemistry has been studied by lichenologists for the past hundred years and has found over 800 secondary metabolites (Muller 2001; Elix 1996). The lichens are rich in polysaccharides, certain enzymes and some vitamins and thus chief source of food for invertebrates and also used as food by man during famines. Species of *Parmelia* are used as curry powder in India. Reindeers (a kind of deer) eat *Cladonia rangiferina* (Reindeer moss) as their main food in polar countries. *Cetraria islandica*, species of *Stereocaulon*, *Evernia*, *Parmelia* and *Lecanora* are used as fodder for different animals.

Some lichens are medicinal and are used for the treatment of different diseases like jaundice, fever, diarrhea, epilepsy, hydrophobia and various skin diseases from very early times. Some lichens are used in leather industry. The astringent substance from thallus of *Cetraria islandica* and *Lobaria pulmortaria* is extracted and used in tannin industry. Natural dyes are also prepared from lichens. Due to the presence of

aromatic substances in the thallus, lichens are used in the preparation of various cosmetic articles, perfumery goods, dhoop, and hawan samagris. Lichens are also mentioned as of medicinal imporatance in ayurveda. ‘Chharila’ which comprises three species of *Parmelia* i.e. *P. perlata, P. perofrata* and *P. sanacti-angeli)* is sold in Indian markets. This drug has astringent, laxative, resolvent, carminative properties and is also supposed to have aphrodisiac property. The drug is considered to be useful for the treatment of diseases like dyspepsia, spermatorrhoea, amenorrhoea, calculi, diseases of blood and heart, stomach disorders, enlarged spleen, bronchitis, bleeding piles, leprosy, scabies, soreness of throat, excessive salivation, tooth-ache and general pain. It is believed that headache is relieved by the smoke of ‘Chharila’. The powdered drug is also applied to wounds, besides a good cephalic snuff.

Literature Review:

Lichens of India are being studied since 1753 AD. Linnaeus (1753) mentioned the occurrence of *Lichen fuciformis* (L.) DC. (*Rocella montagnei* Bel.) from India, in his masterpiece *'Species Plantarum'.* Acharius (1810, 1814) the father of Lichenology described four species, namely *Alectoria arabum* Ach., *Collemarottleri* Ach., *Isidium bassiue* Ach., and *Porina subcutunea* Ach. from India in his classical works *'Lichenographia Universalis'* and *'Synopsis Methodica Lichenum'*. There after the Indian lichens have been described by various lichenologists. About a decade later, Fries (1825) described five new species: *Parmelia cirrhata* Fr., P. *confluens* Fr., *Trypethelium pruinosum* Fr., T. *superbum* Fr., and *Usnea dichozoma* Fr. from Indian continent. Collections of lichens made from Pondicherry and Coromandel Coast were described by Belanger (1838) and comprised forty taxa including six new species (*Gyalecta tropica* Bel., *Parmelia coccodes* Bel., *Ramalina berterii* Bel., *Opegrapha streblocarpa* Bel., *Roccella montagnei* Bel. and *Thelotrema crateriforme* Bel). At almost the same period, Perrottet’s collection of lichens from Nilgiri mountains were described by Montagne (1842) which comprised thirty seven species, two of which were new to science. Some of the conspicuous foliose and fruticose lichens collected by Wallich from eastern India and Nepal were described by Taylor (1847) and described twelve new species. In the same publication, Taylor also included another five new species from Madras and Ceylon the specimens of which were collected by Wight. Out of these seventeen taxa, *Alectoria virens* Tayl., *Parmelia cristifera* Tayl., *P. cylindrophora* Tayl., *P. diademata* Tayl., *P. nepalensis* Tayl., *Stictu Wallichiana*

Tayl., and *Usnea pectinata* Tayl. are now known to be widely distributed. The lichen collections of Strachey and Winterbottom from Kumaon (N.W. Himalayas) during the years 1847-48 were described by Babington (1852). Out of the forty four taxa reported five species were new to science. The lichens collected by Hooker from eastern Himalayas and Nepal during 1846-48 were examined, described and incorporated in '*Synopsis Methodica Lichenum'* by Nylander (1860, 1863). Majority of the species reported were foliose and fruticose in nature. Out of a total of eighty taxa reported, half belonging to the genera *AleIctoria, Cetraria, Leptogum, Parmelia, Stereocaulon, Umbilicaria* and *Usnea* were new. This was an important contribution to considerably enhance the knowledge of the lichens from the Himalayan region.

Collections of lichens were made by Kurz from the Botanic Garden, Calcutta; from Bengal and 24 paragannas; and from the Andamans. All of these were described by Nylander (1867, 1869, 1873), and comprised fifty species (nineteen new) for Calcutta, twenty two species (eleven new) for Bengal, and sixty three species (forty six new) for the Andamans. These collections had a large number of new species and a conspicuously high percentage of the crustose lichens. The genera *Arthonia, Graphis, Lecanora, Lecidea, Opegrapha, Platygrapha, Thelotrema, Trypethe lium* and *Verrucaria* were richly represented. A monotypic genus *Pyrgidium* (*P. bengaliense Krempelh*) of the family caliciaceae was also created. At almost a simultaneous period, Thwaites made extensive collections of the lichens from Ceylon. These were described by Leighton (1869) and comprised of one hundred ninety six species, of which forty four were new, and described a new genus *Asteristion leighta*. Almost three decades later, Nylander (1900) described one hundred fifty nine species including forty eight new species of lichens from Ceylon that were collected by Almquist in 1879. In both the contributions on lichens from Ceylon, the crustose forms predominated. The members of the pyrenocarpeae and graphidineae constitute about one third of the total lichen flora of the island. In a more elaborate work on the Extra-European lichen, Hue (1898, 1899, 1900a, 1901) recorded eighty four taxa from India. Three new species of *Parmelia* from Nilgiri were also described. In another publication, Hue (1900b) enumerated thirty seven species of lichens collected by Gray from Coonoor in Nilgiri Hills. Lichens from the different parts of the country and belonging to the herbarium of E. Levier were enumerated by Jatta (1902, 1905) and comprised one hundred forty two taxa. A few years later, Jatta (1911) enumerated forty six taxa of lichens collected by Luthi from Malabar, and by Long and Gollan from the Himalayas. The same year,

five species were recorded from Ceylon by Zahlbrucker (1911). Thirty one taxa of lichens collected by Somervelie from the high altitudes in the region of the Everest during the Everest expedition in 1924 were reported by Paulson (1925). Smith (1926) described a new family Cryptotheciaceae (a family of primitive lichens), comprising genera *Cryptothecia* and *Stirtonia* based on the material collected from India.

Lichens from the Himalayan region were collected by Kashyap, Chaudhary and Chopra and their collections were determined by Smith (1931) and comprised seventy six taxa. Based on a part of the same collection, Zahlbrucker (1931) established a new genus *Chaudhuria* with a single species *C. indica.* Apparently based on these determinations, Chopra (1934) published an account of lichens from the Himalayas, which comprised seventy five species. Rasanen (1950, 1952) described forty three species of lichens based on the collections of Awasthi made during 1935-1940. Awasthi (1965) recorded the occurrence of about 1300 lichen species from Indian subcontinent. Awasthi (1973) reported a new species *Heterodermia himalayensis* from the Pithoragarh area of Kumaun zone. Awasthi (1975) documented the floristic account of Pindari Glacier catchment area from Kapkote to Pindari Glacier and enumerated one hundred and twenty two species of lichens belonging to thirty eight genera spread over eighteen families. Further a new species of *Leptogiuma skotense* was described by Awasthi from Kumaun (Awasthi & Akhtar, 1977). Awasthi (1980) described new variety of *Pyxine* from Kumaun area.

Pant & Upreti (1993) during revision of the genus *Diploschistes* reported occurrence of eleven species and one type species from the Kumaun Himalaya. Upreti & Aptroot (1996) described a new species of *Lithothelium himalayense* from the Uttrakhand. Awasthi (1988, 1991, 2007) keyed out both micro and macro lichens from different regions of India. Pant (2002) enumerated two hundred and three lichen species belonging to sixty four genera and thirty two families from the two hot spots of Kumaun Himalayas. Upreti *et al*. (2003) studied the genus *Phyllopsora* and reported five species from Kumaun zone. Joshi *et al.* (2007) enumerated eighty two species of lichens from Khuliya Top area of Pitthoragarh district. Joshi *et al.* (2008) enumerated one hundred thirty six lichen species from Pindari Glacier valley. Prasher & Chander (2008) studied ethnobotanical uses of sixteen lichen species of Nanda Devi Biosphere Reserve. Joshi (2010) enumerated three hundred ninety four species belonging to ninety four genera and forty one families from alpine region of Kumaun Himalaya. Mishra *et al*. (2010) enumerated three hundred ninety one species of lichens belonging

to ninety six genera and forty four families from Pithoragarh district of Kumaun Himalaya.

Mishra *et al*. (2011) revised the genus *Phyllopsora* from India and described new species *Phyllopsora catervisorediata* from Kumaun Himalaya. Mishra (2012) studied the distribution of lichens in Kumaun region of Uttrakhand and recorded the occurrence of six hundred thirty species belonging to one hindred thirty six genera and forty four families. An account of lichen diversity of Kargil district of Ladakh region was given by Rahim *et al.* (2013), in which twenty five species of lichens belonging to thirteen genera and eight families were enumerated. Lichens have also been reported from Leh region of J&K (Kumar *et al*. 2014). An updated and complete checklist of three hundred sixty five lichens belonging to thirty five families and ninety one genera of Jammu and Kashmir was published by Goni & Sharma (2015). They also reported occurrence of forty four species belonging to twenty genera and eighteen families as new addition to the lichens of the J&K state. Chander (2015, 2016) reported one hundred twenty eight species of lichens belonging to 43 genera (*Aspicilia, Buellia, Bulbothrix, Caloplaca, Candelaria, Canomaculina, Canoparmelia, Chrysothrix, Cladonia, Collema,Dermatocarpon, Diploschistes, Dirinaria, Evernia, Everniastrum, Flavoparmelia, Flavopunctelia, Graphis,Heterodermia, Hypotrachyna, Lasallia, Lecanora, Leptogium, Lobaria, Parmelia, Parmotrema, Peltigera, Pertusaria, Phaeophyscia, Physcia, Porpidia, Punctelia, Pyxine, Ramalina, Rhizocarpon, Rhizoplaca, Rimelia, Staurothele, Stereocaulon, Sticta, Umbilicaria, Usnea, Xanthoparmelia* and *Xanthoria*) from Nanda Devi Biosphere Reserve and Valley of Flowers National Park, India. Mishra & Upreti (2016) studied the diversity and distribution of macro lichen in Kumaun Himalaya, Uttarakhand and recorded the occurrence of two hundred forty six species of lichens belonging to forty five genera and thirteen families. Prasher *et al.* (2016) recorded three species of *Cladonia* (*C. bacillaris, C. floerkeana* and *C. praetermissa* var. *praetermissa*) as new reports from North India. Devkota *et al.* (2017) studied the Distribution and national conservation status of the lichen family Lobariaceae (Peltigerales) from subtropical luxuriant forests to the alpine scrub of Nepal Himalaya. This study summarized two genera *Lobaria* and *Sticta,* each with seven and six species respectively, reported from ten different districts of Nepal. *Lobaria adscripturiens*, *L. fuscotomentosa*, *L. affquercizans* and *S. limbata* were new records for the lichen flora of Nepal.

India has a rich diversity of lichens as represented by 2450 species belonging to 305 genera and 74 families, which is about 10% of the 20,000 lichen species reported from world over (Singh & Sinha 2010). Present estimate of lichen representation in India needs to be reassessed as many more areas remain unexplored (Negi & Gadgil 1996; Negi & Upreti 2000). Maximum lichen diversity in India is reported from South India and Eastern Himalaya (Awasthi 2000). Western Himalyan lichenogeographical regions accounts for 812 taxa of lichens including 781 species of which 67 (8.3%) are endemic species (Nayaka & Asthana 2014).

Himachal Pradesh is one of the lichen rich regions of Himalayas (a hot spot of lichen diversity in India). Awasthi & Hoeg had collected a large number of lichens from Kangra, Takshi, Chandra, Kullu, Pin and Spiti valley of this area and published an account of lichens from Shimla, Spiti and Chandra valley in 1953, respectively (Awasthi 1953; Hoeg 1953). Sharma & Sharma (1991) studied the lichen of Mussoorie and Shimla hills with notes on saxicolous taxa of Lahaul and Spiti. Upreti (1999) explored Greater Himalayan National Park (GHNP) of Kullu district in Himachal Pradesh. He collected 600 lichen specimens from Jiwanal, Sainj and Thirthan valley of the park and described one hundred ninety two species. Presence of 192 species belonging to 65 genera and 31 families of lichens in GHNP, which is a comparatively smaller area of Himachal Pradesh, clearly proves the lichen richness of the state. Sharma & Sharma (2000) studied the biodiversity of lichens of the high altitude from the Himalayas. Nayaka & Upreti (2000) studied seventeen species of lichens from the Mandi District. Sharma *et al*. (2001) give an overview of lichens of Himachal Pradesh. Nayaka *et al*. (2002) reported lichens from Solan district, Himachal Pradesh. Sharma *et al.* (2002a) studied lichen flora of from Sirmaur district. Sharma *et al.* (2002b) studied lichens of Sangla valley (H.P.). A study on lichens of Sirmaur District was published by Upreti *et al*. (2002) in which 112 species belonging to 26 families & 44 genera were recorded. Srivastava *et al*. (2004a) studied lichen from Shimla District. Srivastava *et al*. (2004b) studied the lichen of Bilaspur, Hamirpur and Una district of Himachal Pradesh, India. This study revealed the occurrence of 70 species belonging to 36 genera and 23 families. Sharma & Kaur (2005) studied temperate species of lichens from Chail. Srivastava (2005) studied the distribution, diversity and pollution status of lichens in Great Himalyan National Park, Kullu. Yadav (2005) documented 503 species of lichens from Himachal Pradesh. These include 25 species of lichen

belonging to 14 genera and 13 families from Mandi district. Prasher & Chander (2005) reported 30 species from Kullu district of Himachal Pradesh.

Research Gap Identification:

The detailed review of published records of lichen reveals that whereas, a lot of exercises to explore lichen of India, North Western Himalaya and Himachal Pradesh have been carried out by various workers, however diversity of lichen in Mandi is data deficient and understudied. Only forty species of lichens have been reported so far from some localities of Mandi district (Nayaka & Upreti 2000; Yadav 2005, Thakur & Chander 2018). There are very least studies pertaining to diversity, mapping of distribution, status and substrate relationship, economic and ecological importance of lichen in the study area.

Objectives:

1. Enumerate the lichens growing in the different habitat/forest type in Mandi.
2. Identify indicator species of lichens with respect to health of the forest.
3. Study the lichens being utilized by the ethnic communities or the local people in the district.
4. Carry out biomonitoring studies at some localities of the study area.

Proposed Methodology:

Study area: The study area for the proposed research will be Mandi district of Himachal Pradesh (Figure 1). It is located in sub-tropical climate zone of Shivalik hills in Western Himalayas. The total geographical area of Mandi district is 3950 km^2, of which 373 km^2 is under forest cover. Sikandar Dhar, Ghugad Dhar and Dhar Kote are some of the major mountains. Its average elevation is 850 meters (2,790 feet). Temperatures usually range between 6.7^0C to 39.6^0C with an average 1679 mm annual precipitation. The major vegetation of the study area comprises of sub-tropical pine forest (*Pinus roxburghii, Terminalia chebula, Pyrus pashia, Syzygium cumini, Emblica, Acacia catechu, Murraya*), ban oak forest (*Quercus leucotrichophora, Rhododendron arboretum, Pinus roxburghii, Pinus wallichiana, Lyonia ovalifolia, Cedrus deodara*), western mixed coniferous forest (*Rhamnus purpureus, Pyrus lanata, Acer caesium, Berberis aristata, Aesculus indica, Quercus dilatata, Cedrus deodara, Taxus bacata, Picea smithiana*), moist temperate deciduous forest (*Pinus wallichiana, Abies pindrow, Picea smithiana, Leucocoprinus*), kharsu Oak forest (*Quercus semecarpifolia, Quercus leucotrichophora*), sub alpine forest (*Abies pindrow, Pinus*

wallichiana, Juniperus, Quercus semicarpifolia, Rhododendron, Berberis, Salix, Betula utilis), alpine pasture (*Withania somnifera, Plectranthus rugosus, Salix elegans*), West-Himalayan upper oak-fir forest (*Quercus semecarpifolia, Rhododendron, Betula alnoides*), moist deodar forest (*Pinus wallichiana, Cedrus deodara, Quercus leucotrichophora, Berberis lyceum, Clematis Montana*) and moru oak forest (*Quercus semecarpifolia, Quercus leucotrichophora, Betula alnoides*). Major tree species found in these forests are blue pine (*Pinus wallichiana*), deodar (*Cedrus deodara*), silver fir (*Picea pindrow*), spruce (*Picea smithiana*), chil (*Pinus roxburghii*) and various species of oak (Champion & Seth 1968). The protected areas of the district are Bandli Wildlife Sanctuary, Nagru Wildlife Sanctuary and Shikari Devi Wildlife Sanctuary.

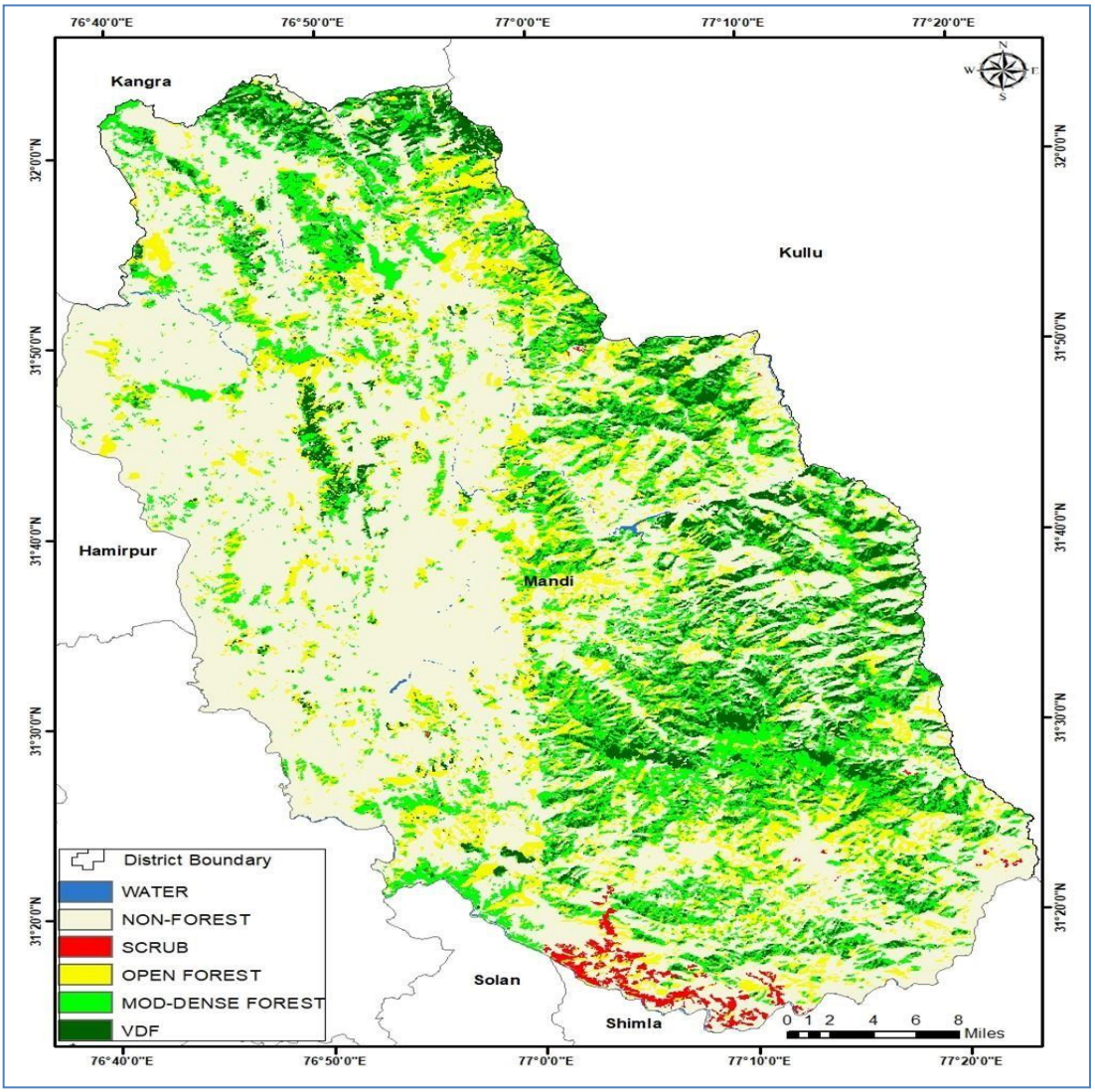

Figure 1: GIS map of district Mandi Himachal Pradesh, India

(Source: HP State Council for Science, Technology and Environment)

Collection of specimens: In the field, both micro and macrolichens can be seen through naked eyes. A hand lense (10X) will be used to check the structure of thallus while collecting the lichens. Efforts will be made to collect mature specimens and in adequate quantity. A Sharp knife, flat edge chisel and a hammer (weight of 1 to 2 kg), paper bags, labeling stickers, a field note book, pen, pencil and vasculum will be used during field collections. The details of locality, substrate, host plant, altitude, collection number with date and macroscopic characters of specimen will be recorded in the field note book during excursions (Mueller 2004). Fresh specimens will be observed and sun dried at the camping site. These will then be brought to laboratory for further taxonomic studies and will finally be deposited in CPUH (The Herbarium, Department of Bio-Sciences, Career Point University Hamirpur) after confirming identity.

Identification of Specimens: The collected lichen specimens will initially be segregated according to their growth forms and further grouped according to fructification type (apothecia, perithecia, sterile). The identity of specimens will be confirmed by comparing their specific morphology, anatomy and chemistry with authenticated taxonomic keys (Awasthi 1991, 2007; Purvis *et al.*1992; Goward 1994a,b; Divakar & Upreti 2005, Rai & Upreti 2014a, b).

Macro morphology of lichen samples will be studied under dissection microscope. The texture of the thallus (smooth, rough), presence of finger like projections (Isidia), granular structures (Soredia), black spots (Pycnidia), white decomposition area (Pseudocyphellae), the morphology of lobe, hair like structure (cilia), length of thallus, the branching pattern, the cylindrical flatness of the thallus, colour of lower surface, the presence of any pores (cyphaellae, pseudocyphellae), presence or absence of rhizines (root like structures), their color, distribution, branching, and abundance. Microscopic observations will be recorded by using light microscope (Prasher & Chander 2006). All the measurements will be taken in 5% KOH solution. Dried specimens will be revived in 2% KOH. Common mountants/stains to be used for microscopic study include lactophenol, cotton blue (for studying cyanophilly of spores and mycelium), 1% Phloxine (for anatomical studies and to determine the presence or absence of clamps and for measuring hymenial elements and hyphae), 50% gycerine (used as mountant to make permanent slides and to remove excess stain), Melzer's reagent (to study blueing of the ascus tip) and congo

red (to stain ascus wall, paraphyses, ascospores & excipular tissues). Fluorescence of lichens will be examined by placing them in a closed UV lamp chamber and exposing UV light with 254 and 365nm wavelengths. The lichen substances will be identified either by using chemical color spot test or micro-crystallography or TLC or HPLC.

Chemical Spot Tests (Culberson 1969; White & James 1985; Orange *et al*. 2001): For K test, 10-25 % aqueous solution of potassium hydroxide will be applied with the help of surgical syringe to cortex, medulla, and parts of apothecia; for C test, a freshly prepared aqueous solution of calcium hypochlorite or bleaching powder or modern commercial bleaching fluid will be used; for KC test, K immediately followed by C will be applied; for Pd test, 1-5% solution of para-phenylenediamine will be prepared in a small amount of ethanol (70-95%) for one day use as it is unstable and cannot be used for the next day. If required, a more stable solution (Steiner's solution) will be prepared by dissolving 1.0 gm of para-phenylenediamine and 10gm of solution sulphite in 100 ml of distilled water with 1.0 ml of a liquid detergent.

Micro-crystallography (Asahina and Shibata 1954): Small pieces of lichen specimen will be placed in the center of microslide and chemical substances present in it will be extracted on the slide by dropping acetone on it with the help of dropper. Drops of acetone will be small and each drop will be added after the evaporation of the previous drop. This gives the crystal dark white rings around the lichen pieces. Then the lichen pieces will be removed gradually and a drop of crystallization reagent will be inserted above the thick crystal dark white rings produced around the lichen pieces. After placing a cover slip on the crystallization agent, the slide will be gently heated over the spirit lamp and then will be allowed to cool down and make crystals. Then the slide will be observed under compound microscope and the lichen substance will be identified based on the shape of the crystals.

Thin Layer Chromatography (Culberson 1969; Culberson & Kristinsson 1970): Many lichen substances are undetectable in color. In such cases, TLC will be carried out. In TLC various lichen substances present in a lichen thallus get separated as spot on TLC plates. These spots will be identified with the help of lichen TLC manuals (Culberson 1972a). The lichen substances will be extracted by digesting thallus piece with acetone in small test tube and acetone extract will be obtained. Silica gel pre-coated thin aluminum plates will be used and a line will be drawn at 2 cm from base of the plate (loading line) and another at 15 cm (finishing line). Many spots on the 2 cm line will be marked at equal distances. The number of spots should be consistent with

the number of test tubes of samples, which are required to be checked for chemistry. The minimum distance between two spots should be 0.8 cm. Spots on low loading lines will be overlapping of spots developed. According to the number of requirements and samples, the width of the plate can be cut in different sizes. Acetone extract in the test tubes will be loaded on the TLC plate with the help of capillary tube. This plate will then be placed in TLC tank containing required solvent system containing toluene, dioxane and acetic acid in the ration 180:45:5 will be used. However if required other solvent systems can also be used. The solvent rises up on the TLC plate passing through the loaded spot. The heavy lichen substance within the spot settles down near the base of the plate, while the mild substances will be removed because the solvent rises upwards. The solvent will be allowed to touch the finishing line drawn at 15 cm and then to receive out of the TLC tank. Lichen substances separated on the TLC plate are usually invisible or colored. They appear more by spraying the color reagent and heating. For this, 10% sulphuric acid solution will be sprayed over the TLC plate. After the spraying, the TLC plate will be placed in a hot air oven (110^0C) for a few minutes. TLC plate will be pulled out of the oven after 3-5 minutes. The spot appearing on the TLC plate will be identified as per their color and the distance they travelled from the loading point. The distance travelled by lichen substance (spot) which is referred to as Rf class, will be calculated as follows:

Rf value = (distance travelled by substance (spot) distance travelled by solvent) × 100

Rf class = divide TLC into approx.7 equal parts from the loading line to the last spot (atranorin), each division is a Rf class.

Observation of TLC plate under shortwave UV lamp is sometimes necessary before and after heating. The spots appear differently while some spots emit fluorescence and such spots are marked with pencil. These are added characters for identification of lichen substances. So wherever required, TLC plate will be observed under shortwave UV lamp. For identification of fatty acids, TLC plate will be sprayed with distilled water. Fatty acids appear as oily, grey spots on drying of water. These will be circled with dotted lines using pencil. After this step, sulfuric acid solution will be sprayed and then the plate will be heated. The fatty acid spot does not give any color after heating. Fatty acids will be also identified on the basis of their Rf class or Rf value.

High performance liquid chromatography (Culberson 1972b): In general, authentic samples of lichen substances will be used when available, and each

compound will be injected separately to determine its retention index (RI). Otherwise, herbarium specimens with established chemistry will be used as standards. In these cases 10 mg of lichen material will be extracted in 1ml of cood acetone for 60 minutes. A Kontron HPLC system connected to a data system 450 instrument with a 430 UV detector and a 360 auto-sampler will be used. A spherisorb ODS2 column (Kontron), 5 μm, 250X4.6 mm, at room temperature will be used. Two solvent systems will be employed. Solvent system A will be Aqua bidest containing 1% orthophosphoric acid and solvent system B will be 100% methanol. The solvents will be degassed for 30 min in an ultrasonic vibrator prior to use. The substances will be dissolved in acetone to which the two standards will be added (20 mg of benzoic acid and 20 mg of solorinic acid per 1000 ml of acetone). Benzoic acid elutes very rapidly and solorinic acid elutes slowly. The run will be accomplished with 30% of solvent system B and continued isocratically for 1 minute at a flow-rate of 0.7 ml/minute. After 1 minute, 20 μl will be injected and solvent system B will be increased to 70 % within 14 minutes, then up to 100 % B for a further 18 minutes. At the end of the run the solvent system B will be decreased to 30 % B for 16 minutes before a new run will be started. The compounds will be detected at λ = 245 nm and UV spectra (λ = 200-400 nm) of each peak eluted. Benzoic and solorinic acids will be used as internal standards by their addition to the extraction liquid (acetone). Bis-(2-ethylhexyl)-phthalide will be employed as a second internal standard. The RI value of an unknown peak will be calculated as follows:

RI = Rt of peak- Rt of benzoic acid X 100 / Rt of solorinic acid

Preservation of specimens: The dried lichen specimens will be pasted on thick, hard paperboard of dimension 6.5 X 4.5 inches and then placed inside herbarium packet bearing label mentioning herbarium accession number (CPUH), name of species, date of collection, name of locality, altitude, host/substrate and name of collector. The same accession number will be marked on board. Small or delicate samples will be kept within a paper packet pasted to the board, or in small boxes. A piece of tissue paper will be pasted on the board while keeping it inside the herbarium packet. A herbarium packet will contain only samples related to single species and the mixture of samples will be avoided. In case of large quantities of specimen material, specimens will be distributed in two or more packets to prevent the spoiling of the sample due to crushing of the packet**.** Many herbarium packets will be placed directly in the drawers

or cupboard/ hardboard boxes (8 X 5 X 12 inches). Herbarium storage space should be dry to prevent the growth of fungus on lichens**.**

Biomonitoring Studies (Nylander 1866): For the Biomonitoring studies, the lichen thalli (approximately 5g f.wt.) will remove from the bark with sharp knife. The samples will be oven dried for 12h to a constant weight at 90 C. The dried lichen sample (three replicates) will be grind to powder (1.0g each) and digest in the mixture of concentrated HNO_3 and $HCIO_4$ (V/V 9:1) for 1h. Residue will be filter through Whatman Filter paper no. 42 and dilute to 15ml with double distilled water. Analysis will be done with Flame Atomic Absorption Spectrophotometer.

Thesis Outline: Thesis will comprise seven chapters as:

Chapter 1 (Introduction): It will include general information about the diversity, growth forms, host-substrate relationship, economic importance, ecological importance, status and distribution of lichens. It will also include objectives of the study and the research gaps that originated the need for the present study.

Chapter 2 (Literature Review): It will deal with the detailed review of the literature concerning earlier and recent finding by various lichenologists on diversity and distribution of lichens in the World, India and Himalaya with particular reference to North Western Himalaya, Shivalik Himalaya and Himachal Pradesh.

Chapter 3 (Materials and Methods): It will provide the details about the general geography, climate and vegetation of the study area. It will also include details about methodology of collection, field studies/observations, morphological investigations, anatomical studies, identification and preservation of species.

Chapter 4 (Observations): In this chapter all the species will be described morphotaxonomically and arranged alphabetically in the order: family-genus-species. The taxonomic key to order, families, genus and species will be presented for identification of lichen. The following data will be presented for each taxa:

- Brief description of order and family along with authority, citation and distribution.
- Taxonomic key to the genera.
- Brief description of genus along with authority, citation, distribution, status, economic, ecological importance and remarks (if any).
- Taxonomic key to the species.
- Morpho-chemotaxonomic description of species along with authority, citation, herbarium accession number, substrate, locality, date of collection, distribution, status, economic, ecological importance and remarks.

- Micrograph and image of species.

Chapter 5 (Results and Discussion): It will include the results of analysis of diversity studies, lichen-substrate relationship, assessment of status (threatened, rare, endangered, and common) of species, documentation of economic and ecological value of species and distribution mapping of each species.

Chapter 6 (Conclusion and Summary): Comprises summary of entire study according to objectives along with conclusions. It will include inventory of recorded species along with their distribution and threat status. The scope of further research will also be discussed.

Chapter 7 (References): For citation of author in text and for presenting references, guidelines of 'The Lichenologist', which is a Journal of British lichen society (https://www.cambridge.org/core/journals/lichenologist/information/instructions-contributors) will be followed.

Research Plan Schedule:

Research Activity	Progress in Months											
	3	6	9	12	15	18	21	24	27	30	33	36
Course work and extensive literature survey					Jan 2018 - Dec 2018							
Research gap identification and design of methodology					June 2018 - Nov 2018							
Submission of Synopsis for proposed research							Jan 2019 - June 2019					
Field Survey	Jan 2018 to Sept 2020											
Morpho-chemo-taxonomic investigations of specimens		April 2018 – Sept. 2020										
Preservation and deposition of specimens in CPUH			Jan 2020 to October 2020									
Manuscript preparation for publication and Thesis writing					April 2020 to Dec 2020							
Pre-Thesis presentation							September 2020					
Submission of Ph.D. Thesis								December 2020				

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